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Author(s)	Anne C. Jacobs (Allegheny College), Jeanne M. Fair, Marlene Zuk
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Coloration, paternity, and assortative mating in western bluebirds

Anne C. Jacobs[1](corresponding author), Jeanne M. Fair[2], Marlene Zuk[3]

1. Department of Biology, University of California, Riverside, CA 92521
2. Los Alamos National Laboratory, Environmental Stewardship, MS K404, Los Alamos, NM 87545
3. Department of Ecology, Evolution and Behavior, University of Minnesota, Saint Paul, MN 55108

Corresponding Author

Anne C. Jacobs

Department of Biology

Allegheny College

Meadville, PA 16335

Email: ajacobs@allegheny.edu

Phone: (310) 365-8130

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Abstract

Coloration in birds can act as an important sexual signal in males, yet in many species both sexes display bright colors. Social selection may account for this pattern, with more brightly colored individuals pairing together on the best territories. Mutual mate choice may also explain this, as males investing a great deal of parental care in the offspring should be choosy about their social mates. It is less clear whether this pattern of mate choice can apply to extra-pair partners as well. We examined western bluebirds (*Sialia mexicana*) to determine if more colorful individuals tended to pair with one another, both in social pairs and between females and their extra-pair partners. Both male and female western bluebirds display both UV-blue structural plumage and a melanin-based chestnut breast patch, although females are duller than males. Social pairs mated assortatively with regard to UV-blue brightness, but not chestnut coloration. There was no evidence that extra-pair partners mated assortatively, but males with brighter UV-blue coloration had fewer extra-pair offspring in their nests. Older males were more successful at siring extra-pair offspring, despite displaying no differences in coloration compared to younger males. Coloration did not play a role in determining extra-pair male success. These results suggest that coloration plays a role in the formation of social pairs, but not mate choice for extra-pair partners.

Keywords: Assortative mating, coloration, extra-pair paternity, mate choice, *Sialia mexicana*

Introduction

In many species of birds, females as well as males display brightly colored plumage (Amundsen 2000). Sexual selection has typically been invoked to explain such traits in males (Darwin 1871; Andersson 1994); however the selective forces driving the evolution of such coloration in females remain debated (Amundsen 2000; Kraiijeveld et al. 2007; Clutton-Brock 2009). Several hypotheses for female ornaments have been proposed. These include genetic correlation, which suggests female coloration arises as a result of sharing genes for bright plumage with males, in whom plumage coloration evolves under sexual selection (Amundsen 2000; Cardoso and Mota 2010). Under this scenario, selection acts only on males, and brighter females do not necessarily have an advantage compared to duller females.

In contrast, several adaptive explanations of female ornamentation have also been proposed. Female ornaments could evolve under social selection and serve to signal competitive ability to conspecifics (West-Eberhard 1983; Clutton-Brock 2009; Tobias et al. 2012). Mutual mate choice, in which both males and females are selective about their breeding partners, also provides an explanation for female ornamentation (Amundsen 2000). Under both social selection and mutual mate choice, female coloration acts as an indicator of female condition and/or quality, either to competitors or to prospective mates. Females could also adopt male-like coloration as a means of avoiding harassment (Kraaijeveld et al. 2007).

One of the predictions of mutual mate choice is that preference for highly ornamented individuals should lead to assortative mating, in which females bearing better ornaments pair with males bearing better ornaments. Social selection can also produce

this pattern, although by different mechanisms. For example, if ornaments signal the ability to drive off competitors, highly ornamented males and females may end up together because both occupy the best territories. Alternatively, males could compete for the best territories and females could compete amongst themselves for access to the best mate. Thus both mutual mate choice and social selection are expected to produce assortative mating (Kraaijeveld et al. 2007); in contrast, genetic correlation and sexual mimicry would not produce this pattern.

Many species of birds are socially monogamous, and although a single male will pair socially with a single female to rear the offspring, one or both individuals may mate with individuals outside the pair bond (Griffith et al. 2002). Males in socially monogamous species are expected to be selective about their social mates, with whom they share the workload of provisioning the offspring. Whether males should also show a preference when selecting extra-pair mates is less clear. On the one hand, males may not gain much by being choosy about extra-pair partners. If individual copulations are cheap, a male does not invest much in each extra-pair partner, and thus has little reason to be selective (Trivers 1972). However, if opportunities for extra-pair copulations are limited, and if females differ markedly in their ability to rear offspring successfully, one might predict that males will show preferences when selecting extra-pair partners as well. In cases where partners differ greatly in their quality and the cost of searching for a new mate are low, we might expect choosiness in both sexes, even when males invest less in a mating than females (Parker 1983).

Females are predicted to be choosy about all potential mates. However, what characteristics they prefer may differ depending upon what benefits females gain. If

females select mates based on good genes and plumage acts as an indicator of such genes, we expect females to prefer the same traits in social and extra-pair partners. However, if females select social partners based on direct benefits, they may prefer different traits in social and extra-pair partners, as extra-pair males provide few, if any, benefits to the females with which they mate. This may explain why some studies find that females prefer different characteristics in social versus extra-pair mates (Weatherhead and Boag 1995).

We tested the prediction that brightly colored individuals would pair assortatively by examining coloration, mating patterns, and paternity success in western bluebirds (*Sialia mexicana*). Western bluebirds are socially monogamous with reasonably high rates of extra-pair paternity; in some populations, as many as 45% of nests contain at least one extra-pair offspring (Dickinson 2003). Coloration has been implicated as an important sexual signal in closely-related species, including eastern bluebirds (*Sialia sialis*; Siefferman and Hill 2005a, b; but see Liu et al. 2007, 2009), and mountain bluebirds (*Sialia currocoides*; Balenger et al. 2009). Evidence in western bluebirds suggests that females control extra-pair copulations and only mate with preferred males (Dickinson 2001; Ferree and Dickinson 2011). Female western bluebirds can potentially double their reproductive success in a season by producing second broods, and females that produce second broods breed earlier and are heavier, implying that individual quality plays a role in breeding success (Jacobs et al. 2013).

We predicted that more brightly colored males would pair socially with more brightly colored females, indicating that coloration plays a role in mate choice and/or social competition in this species. Assortative mating in social pairs has been documented

in other passerines (Andersson et al. 1998; Griggio et al. 2005; Bitton et al. 2008; Rowe and Weatherhead 2011), but few studies have attempted to determine whether these patterns also apply to extra-pair paternity. Here, we examine rates of extra-pair paternity in relation to male and female coloration. We predict that, if males are selective about extra-pair partners, then assortative mating by color will occur between females and their extra-pair mates as well, assuming that plumage coloration is used in both social and extra-pair mate choice (c.f. Kraaijeveld et al. 2004). We also predict that bright females that are "mismatched" with their social mates (i.e. bright females paired with dull males) will have greater numbers of extra-pair offspring in their nests.

Materials and Methods

Study species and field methods

Western bluebirds are 23–30 g passerines. They have feathers that reflect in the UV-blue part of the electromagnetic spectrum on their head, back, rump, wings, and tail; these get their color from the feather microstructure (Siefferman and Hill 2003). In addition, they have a melanin-based chestnut patch on the breast (McGraw et al. 2004). Both males and females display similar coloration, although males are brighter (Figure 1). Bluebirds are secondary cavity nesters, and will readily use artificial nestboxes (Guinan et al. 2008), allowing easy access to nestlings for paternity assessment.

We conducted all field work on the Pajarito Plateau in Los Alamos County, New Mexico which has sites with nestboxes on trees placed 1-2 meters off the ground. For further details on the sites and nestboxes, see Fair et al. (2003) and Jacobs et al. (2013). We conducted the project during the breeding season (May through August) in 2010,

2011, and 2012. During that time, we recorded which nestboxes were occupied, the number of eggs laid, the hatching date of the eggs, and the number of nestlings that successfully fledged. We visited each site at least once every two weeks. To determine the hatch date for a given clutch, we estimated the age of the nestlings and counted backwards. Bluebird nestlings hatch synchronously (Guinan et al. 2008), so we assigned a single hatch date to each nest.

After nestlings had hatched, we captured the adults by using mist nets or trapping them in the box. Nets were placed directly in front of boxes, and primarily captured individuals attempting to enter the nestbox. In some cases, we captured individuals twice as part of a different project; once using a box trap, and once using mist nets. In each case, the male captured was the same both times. Genotyping of females and offspring confirmed that the female captured was always the mother of the nestlings in the box; therefore, we infer that males captured by mist net in front of a box were the social fathers. Helping has been documented in other populations of bluebirds (Dickinson et al. 1996) but is quite rare in our population. We captured second males at only 4 of the 76 nests we sampled, and these were usually males in their first year of breeding helping at the nest of an older male. We assigned social paternity to the older male, and this was the male to whom the majority of the nestlings were assigned genetically (see below). We never recorded an instance of helper males siring offspring in the nest at which they helped.

For each adult, we recorded sex and measured wing chord (±1 mm) with a ruler, tarsus length (± 0.1 mm) using digital calipers, and mass (± 0.1 g) using a portable electronic balance, and fitted the bird with a numbered US Fish and Wildlife Service

aluminum band. We aged adult males as either second year (SY – one year old) or after second year (ASY – two or more years old) based on the presence or absence of molt limits (Shizuka and Dickinson 2005). In many cases, we were unable to positively age females, in which the molt limits can appear less distinct than they do in males (D. Shizuka, personal communication), and in a few cases, males began molting near the end of the breeding season and we could not age them reliably. We classified such birds as after hatch year (AHY) and excluded them from analyses using age as a factor. We collected total of 9 feathers each from the breast (chestnut coloration) and the rump (UV-blue coloration) for all adults and stored these in opaque envelopes until color analysis. We then used a sterile needle to puncture the brachial vein and collect 10-50 µL of blood on an FTA® (Whatman Ltd.) card for DNA extraction.

Once nestlings had reached nine days of age or older, we banded them and took a small (10-50 µL) blood sample from each one, which we stored on FTA® cards until DNA extraction and analysis. We then monitored nests until all the nestlings had fledged. Bluebirds in the population typically fledge at 19-21 days, but can fledge as early as 16 days (Fair and Myers 2002; Guinan et al. 2008). If we found nests empty after the nestlings had reached 16 days of age, we counted the nestlings as fledged. If we found a nest empty before that time, we counted it as depredated. In our population, a small percentage of birds produce two successful broods during a single breeding season (Jacobs et al. 2013). For this study, we did not distinguish between broods, but included all nestlings from a given set of social parents for that year in the analysis. All work was done in accordance with the Guidelines to the Use of Wild Birds in Research (Fair et al.

2010) and with the approval of the Los Alamos National Laboratory Animal Care and Use Committee (Protocol #10-60).

Coloration Measurements

We taped nine feathers from each body region on a black, non-reflective background in an overlapping pattern to mimic how they would appear on the birds (Siefferman and Hill 2003). We then measured reflectance from 300-700 nm using an Ocean Optics USB4000 spectrophotometer (range: 200-1100 nm; Ocean Optics Inc., Dunedin, Florida, USA) and a xenon light source. We measured the reflectance of each sample relative to a white standard (Ocean Optics). For each reading, the probe was encased in a black sheath to exclude light and keep the probe at a 90º angle relative to the feathers for all readings. We took five readings of each region by lifting up the probe and then replacing it. These readings were averaged together.

To quantify individual coloration, we used three measures: hue, chroma, and brightness. To measure brightness, we took the average reflectance value across a 300-700 nm spectrum. This was done for both breast brightness (chestnut) and rump brightness (UV-blue). Other papers examining UV-blue coloration in bluebirds have measured the intensity, or the peak reflectance value, instead of brightness (Siefferman and Hill 2003; Balenger et al. 2009). We chose to use average brightness because this measure could easily be calculated the same way for both UV-blue and chestnut plumage. Our measurements of overall average brightness for the UV-blue feathers were highly correlated with the measure of blue intensity ($r = 0.95$, $N = 141$, $P < 0.001$). To measure chroma, we summed the reflectance values over the area of interest (300-500 for UV-blue

region, 500-700 for chestnut region) and divided that by the total summed reflectance over the whole spectrum (Siefferman and Hill 2003). For UV-blue feathers, we calculated the hue as the wavelength of peak reflectance. The chestnut feathers exhibited no obvious peak, and reflectance values increased steadily beyond 700 nm (Figure 1). To calculate hue for this region, we took the wavelength that corresponded to the reflectance value midway between reflectance at 300 and 700 nm, or $\lambda[(R_{max}+R_{min})/2)]$ (Rowe and Weatherhead 2011).

Molecular Analyses

DNA was eluted from the FTA® cards according to the manufacturer's instructions. We checked DNA concentration using a Nanodrop ND-1000 (Thermo Scientific, Wilmington, DE); all samples contained 10-150 ng/µL of DNA. To assign paternity, we used highly variable microsatellite loci previously described for western bluebirds and closely-related eastern bluebirds. We amplified fragments from five loci: Smex5, Smex9, and Smex14 (Ferree et al. 2008) and Sialia36 and Sialia37 (Faircloth et al. 2006). PCR products were sized using an ABI 3100 Genetic Analyzer, and we confirmed product size visually using GeneMapper v4.1. We calculated allele frequencies and assigned paternity to individual males using the program CERVUS 3.0 (Kalinowski et al. 2007). The microsatellites we used had an average of 11.2 alleles per locus, and none of the allele frequencies deviated from Hardy-Weinberg equilibrium. The combined probability of non-exclusion was 0.008 when the mother was known. In cases where offspring showed no mismatches in alleles relative to the social father, we classified them as within-pair offspring. We assigned offspring to extra-pair sires if CERVUS assigned

paternity to a given male with 95% confidence or if CERVUS assigned a male with 80% confidence and the putative father was from the same field site or a field site in close proximity to the nest. We used this criterion because, during the breeding season, most conspecific intruders and extra-pair males come from adjacent territories (Dickinson 2001). In addition to assessing paternity, we also used DNA samples to determine if adults were infected with avian malaria (*Plasmodium* spp. and *Haemoproteus* spp.). To do this, we used a nested PCR reaction following the methods described in Waldenström et al. (2004) to amplify a section of mitochondrial DNA in the parasites.

Statistical Analyses

We performed all analyses in SAS v9.2 (SAS Institute 2008). We checked data for normality using a Shapiro-Wilk test. Breast hue and brightness were non-normally distributed, and we used a square root transformation on the data before analysis. Males and females have different coloration, with females displaying duller, lighter plumage than males (Figure 1). We therefore analyzed coloration in each sex separately in the principal components analysis (see below). Data on UV-blue coloration did not vary among years, and so we pooled data from all years for the analysis. However, we found a significant effect of year on breast coloration variables. To control for this, we standardized data for breast hue, chroma, and brightness within each year, setting the mean to zero and the standard deviation to one.

Color variables (hue, chroma, and brightness) taken from the breast region were highly correlated with each other (Table 1); we performed a principal components analysis to reduce the number of variables. A separate principal components analysis was

246 run on the breast color variables for each sex. The first principal component from each
247 analysis was retained using the Kaiser-Guttman criterion (eigenvalues greater than one,
248 Table 2). Hue, chroma, and brightness for the UV-blue coloration were weakly correlated
249 with each other and we could not easily reduce these variables using a principal
250 components analysis (Tabachnick and Fidell 2007). Instead, we chose to focus on a
251 single measure, brightness, because this measure exhibited the greatest variation between
252 individuals, as demonstrated by a coefficient of variation that was almost an order of
253 magnitude greater than the coefficients for hue and chroma (in males: UV-blue hue CV =
254 0.027, UV-blue chroma CV = 0.052, UV-blue brightness CV = 0.206).
255 Over the course of the study, we captured and banded 129 adult birds; of these, 21
256 (16%) were recaptured in multiple years. If they stayed mated to the same partner in both
257 years (4 pairs), we only included data from the first year in which that pair was captured
258 in the correlation analyses. If an individual acquired a new social mate in subsequent
259 years, we viewed this as an independent pairing event and included data from both pairs
260 in the analysis. To determine the effects of coloration on rates of extra-pair paternity
261 within a nest, we used a generalized linear model with binomial error, using the number
262 of within pair young as the dependent variable, the total number of offspring in the nest
263 as the binomial denominator, and the coloration of both social parents as predictor
264 variables. We tested for an interaction between social father coloration and social mother
265 coloration to determine if “mismatched” pairs had higher rates of extra-pair paternity.
266 To see if coloration acted as an indicator of individual quality, we used a general
267 linear model to test for relationships between male and female color variables and other
268 characteristics, such as mass (which we use as a proxy for condition; Schamber et al.

2009), tarsus length, breeding date (hatch date of the offspring), and infection with avian malaria. Due to limited data on female age, we did not include this variable in our analyses of female coloration as it would have dramatically reduced our sample size. Data on tarsus length and breeding date varied between years, so we standardized these variables within a given year by setting the mean to 0 and the variance to one.

To test whether coloration affected a male's ability to sire offspring in another nest, we used a logistic regression, classifying males as either successful or unsuccessful at fathering extra-pair young. We included mass, male age, breast, and rump coloration, and tarsus length (a measure of body size) in these analyses. We compared coloration of cuckolded males (males with extra-pair young in their nests) and extra-pair males using paired t-tests. In one instance we detected offspring from two extra-pair sires within the same nest. We averaged the coloration scores of these two extra-pair males. In some cases, we collected data from individuals whose partners we were unable to catch or whose nests were depredated before we could take samples from the nestlings. Because of this, sample sizes vary.

Results

We retained the first principal component from the analyses of breast feather coloration in males and females. In both sexes, the first component had high positive loadings on breast hue and breast chroma, and high negative loadings on breast brightness (Table 2). Higher PC1 scores correspond to individuals that have darker, redder, more saturated chestnut feathers on their breast patches. Neither the brightness of the UV-blue plumage nor PC1 of breast plumage differed significantly between second

year and after second year males (UV-blue coloration: $F_{1,56} = 1.06$, $P = 0.31$, chestnut PC1: $F_{1,57} = 0.03$, $P = 0.87$); we did not have sufficient data to test for age differences in female plumage coloration.

Assortative Mating

We obtained coloration data from 48 social pairs in which we captured both the male and the female. Within social pairs, bluebirds mated assortatively with respect to UV-blue coloration, with brighter males pairing with brighter females ($r = 0.31$, $N = 48$, $P = 0.03$, Figure 2). However, the males and females of social pairs did not show any evidence of assortative mating by breast coloration ($r = 0.08$, $N = 48$, $P = 0.57$). The total number of offspring fledged from a nest did not depend on male coloration, female coloration, or the interaction between the two, either when considering rump coloration ($F_{3,45} = 0.52$, $P = 0.67$) or breast coloration ($F_{3,45} = 1.34$, $P = 0.27$).

Coloration and Extra-pair Paternity

We genotyped 324 nestlings over three years, 114 of which we classified definitively as extra-pair offspring (35.2%). Of the 76 nests for which we genotyped nestlings, 51 of them (67%) contained at least one extra-pair offspring. We were able to assign 31 extra-pair offspring to 19 different fathers. One male entered the analysis twice as an extra-pair sire because he fathered extra-pair young in two different years. We had complete coloration data for 20 pairs of females and their extra-pair partners. In one case, a female had extra-pair offspring sired by more than one male; we included both sires in the analysis, but removing one did not substantially affect our results. We found no

315 evidence of assortative mating by coloration between females and their extra-pair
316 partners either when considering UV-blue plumage (r = 0.17, N = 20, P = 0.47) or
317 chestnut plumage (r = -0.07, N = 20, P = 0.77).
318 Interactions between male and female plumage coloration were not significant, so
319 we removed them from the model. Female coloration (chestnut and UV-blue) and male
320 chestnut coloration then had no effect on the proportion of within-pair young in a nest.
321 However, male UV-blue coloration did predict how well a male maintained paternity
322 ($\chi^2_{1,42}$ = 9.13, P = 0.003), with brighter males siring a greater proportion of the offspring
323 in their own nests (Figure 3). When we looked at the characteristics of males that
324 successfully sired extra-pair offspring, the overall model was marginally significant
325 ($\chi^2_{5,42}$ = 9.43, P = 0.09). However, the only variable that significantly predicted success
326 was male age ($\chi^2_{1,42}$ = 4.38, P = 0.04), with breast coloration as a nearly significant
327 predictor ($\chi^2_{1,42}$ = 3.29, P = 0.07). Males that sired extra-pair offspring were older and
328 tended to have higher PC1 scores (darker, redder breasts) (Table 3). We found no
329 difference in coloration between extra-pair males and the males they cuckholded either in
330 UV-blue brightness (paired t_{11} = -0.27, P = 0.79) or chestnut coloration (paired t_{11} = 0.65,
331 P = 0.53). Neither male nor female coloration showed any relationship with mass, body
332 size (tarsus length), infection status, or breeding date, either when we considered the
333 sexes separately or together (all P > 0.2).
334
335 **Discussion**
336 Social pairs of bluebirds had more similar plumage coloration, as has been found
337 in several other studies of passerines (Andersson et al. 1998; Griggio et al. 2005; Bitton

et al. 2008; Rowe and Weatherhead 2011). Liu et al. (2009) found no evidence of assortative mating in the closely related eastern bluebird, but had a smaller sample size. Brighter UV-blue males maintained a higher proportion of paternity within their own nests, implying that coloration may play a role in social mate choice in this species.

Our finding of assortative mating suggests that coloration indicates quality and/or competitive ability in both sexes. However, we cannot determine which mechanism was responsible for producing the mating patterns we found. Either mutual mate choice or social selection could account for our findings. Western bluebirds are secondary cavity nesters, and as such, access to nest sites may be limited, causing competition both with conspecifics (Guinan et al. 2008) and other cavity-nesting bird species (Duckworth and Badyaev 2007). In bluebirds, both males and females defend the nest site (Guinan et al. 2008). Female coloration may act as a signal of female social status and competitive ability to potential competitors more often than it functions in mutual mate choice (Tobias et al. 2012). Male coloration may act in a similar manner, signaling fighting status to competitors and functioning in male-male competition for territories. In pied flycatchers (*Ficedula hypoleuca*), another secondary cavity nester, Alatalo et al. (1986) demonstrated that male coloration was not directly used in mate choice by females, but that females instead selected the male with the best territory, resulting in the appearance of female preference for color. A similar mechanism could operate in western bluebirds, and if females then compete with each other for males with the best territories, this could produce the pattern we found in social pairs. We should note, however, that mutual mate choice and social selection are not mutually exclusive explanations, and that both may operate simultaneously (Kraaijeveld et al. 2007).

The finding that males with brighter UV-blue coloration had fewer extra-pair offspring in their nests suggests that coloration may act as a sexual signal, at least in males. However, eastern bluebird females did not prefer more brightly colored males in either aviary or field experiments, implying that coloration itself is not important in mate choice in this species (Liu et al. 2007, 2009). Instead, coloration correlated with a male's ability to secure a territory early in the breeding season (Siefferman and Hill 2005c). These results lend support to the idea that coloration functions primarily as a social signal in bluebirds, and that assortative mating patterns may result from brighter males and females defending better territories rather than mate choice on ornaments per se.

If bright coloration acts as a signal in territorial male-male competition, perhaps brighter males can better exclude intruders from their territories. This might explain why brighter males maintain a greater share of within-pair paternity. However, we consider male exclusion of competitors to be an unlikely explanation for maintenance of within-pair paternity, as highly aggressive male bluebirds do not have lower numbers of extra-pair offspring in their nests (Duckworth 2006). While it is possible that aggression alone is a poor indicator of social dominance, more aggressive males in this species can better acquire and defend territories (Duckworth 2006; Duckworth and Badyaev 2007), a prediction also made of dominant males.

If brighter pairs hold better territories, one might also predict that such pairs would successfully fledge more offspring. We did not find any evidence that this was the case, but this may be due to factors other than parental quality influencing nestling survival. Predation accounts for a large number of nest failures in our study, and the probability that a nest will be depredated may not depend on individual brightness in this

384 species. A better indicator would be nestling mass prior to fledging. If brighter males
385 hold superior territories, the offspring raised on those territories should be heavier,
386 regardless of paternity. Unfortunately, we lacked consistent data on nestling mass to test
387 this prediction.

388 Our results on how coloration affects patterns of extra-pair paternity are almost
389 opposite the patterns found in mountain bluebirds. There, UV-blue coloration predicts
390 male success at obtaining extra-pair copulations, but brighter males do not have fewer
391 extra-pair offspring in their own nests (Balenger et al. 2009). Mountain bluebirds are
392 congeners of western bluebirds, and one might predict that sexual selection should
393 operate similarly in these species. However, coloration in this species does not indicate
394 parental effort (Balenger et al. 2007), unlike in eastern bluebirds (Siefferman and Hill
395 2003, 2005a). Thus coloration may not play an important role in signaling direct benefits
396 in mountain bluebirds, and would therefore be useless in selecting social mates.
397 Moreover, this study did not take male age into account as a confounding factor, which
398 can influence plumage coloration in some bluebirds (Siefferman et al. 2005, Budden and
399 Dickinson 2009). Female western bluebirds prefer older males as extra-pair partners
400 (Dickinson 2001; Ferree and Dickinson 2011), and extra-pair mate choice for older
401 individuals occurs in our population. Such extra-pair success by older males is common
402 in passerines (Cleasby and Nakagawa 2012), and female choice for overall viability may
403 explain this pattern (Kokko and Lindström 1996). Alternatively, older males' experience
404 may make them better able to exploit opportunities for extra-pair copulations
405 (Weatherhead and Boag 1995).

406 Our data do not suggest any role of female coloration in determining the rates of
407 extra-pair paternity within a nest. In rock sparrows, female coloration used in mate
408 selection has been shown to indicate female quality, specifically the ability to produce
409 second broods (Griggio et al. 2005). In our study, coloration was not linked to either
410 breeding date or mass in females, both of which can indicate double brooding potential
411 (Jacobs et al. 2013). Parker (1983) suggested that if prospective mates differ greatly in
412 their quality, even the sex that invests less in the mating, as extra-pair males certainly do,
413 could display choosiness. However, other models suggest that male investment in extra-
414 pair copulations is so miniscule that even differences in the quality of prospective
415 partners would not lead to male choosiness (Johnstone et al. 1996). Our data would
416 suggest no preference for brighter females on the part of extra-pair males. It is also
417 possible that, if males are selective, they use criteria other than coloration. In many
418 species, the traits important in social mate choice do not necessarily play a role in extra-
419 pair mate choice (Weatherhead and Boag 1995; Kraaijeveld et al. 2004).
420 Our study includes data taken primarily from breeding adults. There may well be
421 floater males (and females) in the population that we did not capture. This pattern
422 resulted from logistical limitations; it is extremely difficult to capture birds reliably
423 without a known point (such as a nest) to which they will return. However, this could bias
424 our results; if brighter males are more likely to hold territories and attract mates, such
425 males would be more likely to be sampled in our dataset. This limitation may also help
426 explain why we found no effects of age on plumage color, in contrast to other studies on
427 bluebirds (Siefferman et al. 2005; Budden and Dickinson 2009). Perhaps only brightly
428 colored second year males can secure territories, in which case our data on second year

males would be biased in favor of brighter individuals. In addition, pairs of bluebirds may have been nesting in natural cavities (rather than nestboxes) at our sites that we did not catch, as well as males whose nests were depredated before we could capture them. This makes assigning paternity more difficult, a problem that can plague studies of wild populations (Hughes et al. 2013). We were unable to assign paternity to a large number of extra-pair offspring, indicating that there were many males present in the population that we did not catch.

In conclusion, coloration plays a role in social pair formation and within-pair paternity rates in western bluebirds, but male age, rather than coloration, determines success at siring extra-pair offspring. This implies that plumage coloration acts more as a signal of quality and/or competitive ability in both sexes.

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569

Table 1: Correlations between color variables

	Rump Hue	Rump Chroma	Rump Brightness	Breast Hue	Breast Chroma	Breast Brightness
Rump Hue	1.0	-0.0001	-0.025	**-0.446****	**-0.264***	**0.371***
Rump Chroma		1.0	-0.011	0.089	0.026	-0.128
Rump Brightness			1.0	0.219	0.147	-0.170
Breast Hue				1.0	**0.492****	**-0.753****
Breast Chroma					1.0	**-0.585****
Breast Brightness						1.0

Values indicate the Spearman correlation coefficients between hue, chroma, and brightness from the breast region (chestnut) and the rump (UV-blue) in male western bluebirds (n=71). * indicates $p < 0.05$, ** indicates $p < 0.001$

Table 2: Results of the principal components analysis performed on chestnut breast coloration variables (hue, chroma, and brightness) in both males and females

	Male PC1 loadings	**Female PC1 loadings**
Hue	0.87	0.90
Chroma	0.73	0.89
Brightness	-0.89	-0.89
Total Variation Explained by PC1	**69.5%**	**80.4%**

We retained the first principal component in each case; all others had eigenvalues less than one.

Table 3: Results of the logistic regression for male success siring extra-pair young

					95% Confidence Interval for Odds Ratio	
Variable	β	Wald Chi-Square	P-value	Odds Ratio	*Lower*	*Upper*
Mass	-0.006	0.001	0.97	0.994	0.687	1.438
Tarsus Length	-0.13	0.109	0.74	0.879	0.408	1.893
UV-blue Brightness	0.0001	0.0003	0.99	1.001	0.895	1.120
Breast Coloration	-0.81	3.285	**0.07**	0.443	0.184	1.068
Age	0.8845	4.378	**0.04**	5.865	1.119	30.749

We considered mass, body size (tarsus length), UV-blue brightness, breast coloration (PC1), and age as predictor variables. The model describes the probability that a male will not sire extra-pair offspring.

Figure Legends

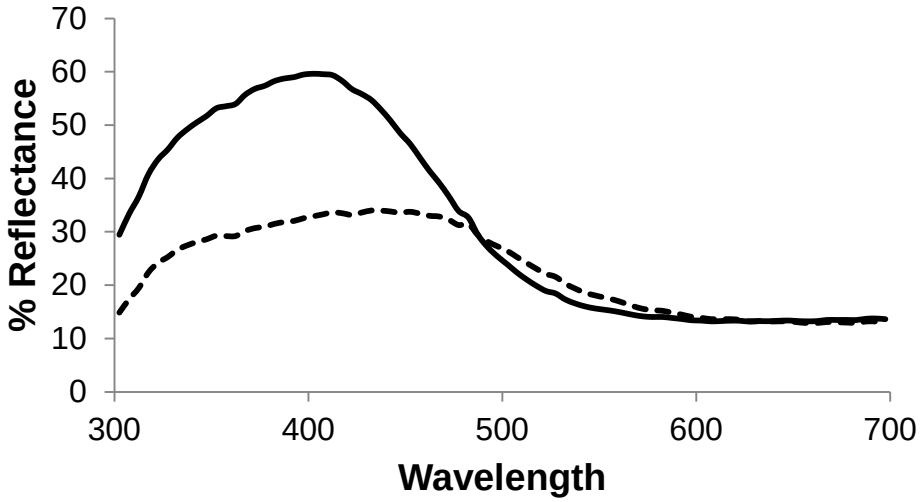
Figure 1: The reflectance curves averaged from birds captured in 2010. The solid line represents the average for males (n = 18) and the dashed line represents the average for females (n = 20). A) The reflectance curve generated by the UV-blue rump feathers. B) The reflectance curve generated by the chestnut breast feathers.

Figure 2: The relationship between brightness of the UV-blue rump feathers in males and females of a social pair.

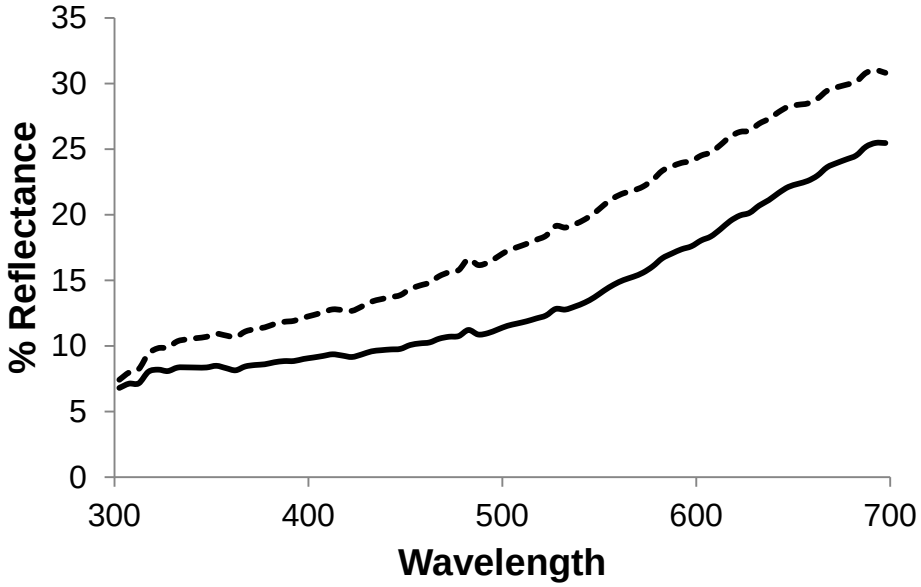
Figure 3: The relationship between male UV-blue brightness and the proportion of nestlings he sired in his own nest.

FIGURE 1

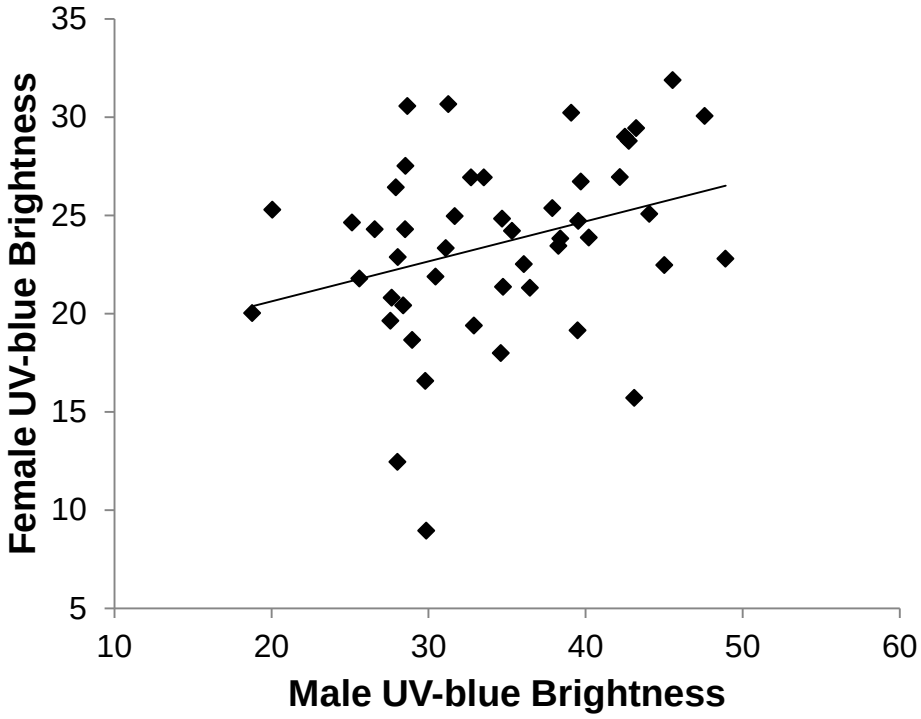
A)


70
60
50
40
30
20
10
0
% Reflectance
300
400
500
600
700
Wavelength

B)


35
30
25
20
15
10
5
0
% Reflectance
300
400
500
600
700
Wavelength

610 FIGURE 2



611

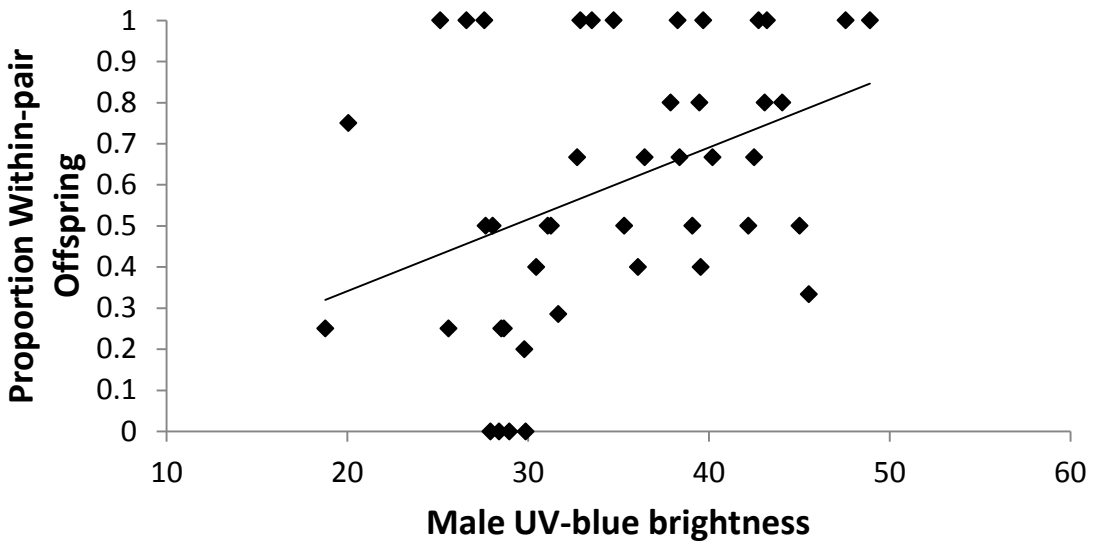
612

613

614

615

FIGURE 3


Proportion Within-pair Offspring
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
50
60
Male UV-blue brightness